Condensed states of a semiflexible copolymer in poor solvent: Figures of eight and discrete size torii

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We examine the condensed states of a simple semiflexible copolymer in which there are two monomer types that are immiscible with each other and with the solvent. Although this is similar to the well known problem of collapse for a semiflexible homopolymer we find that it gives rise to a much richer variety of condensed states. We predict the existence of these states using simple analytic arguments and also observe them directly using Brownian dynamics simulations.

One of the most fascinating physical properties of DNA is its collapse from a diffuse coil to a compact toroidal structure under the addition of condensing agents to the solvent. This phenomenon is not only of biotechnological importance [1, 2] but also serves to illustrate a general principle of competition between bending and solvent energy that is common to the collapse of all semiflexible polymers including other biopolymers, synthetic polymers and also carbon nanotubes [3]. Although much progress has already been made in understanding the kinetics of folding [4, 5, 6] as well as the internal packing arrangements of semiflexible polymer torii [7, 8, 9] much remains that is not understood. Our intention in this paper is to highlight the possibilities that arise when one extends the problem of semiflexible polymer collapse in poor solvent to a copolymer. Inspired by the fact that DNA torii occur as a consequence of a competition between solvent and bending energies we study copolymers with two block types A and B that are immiscible with each other and with the solvent. We begin with an analytic treatment of the problem which leads us to predict several unexpected stable geometries. Using Brownian dynamics simulations we demonstrate that such geometries can self assemble via collapse of a single semiflexible copolymer and that their relative energies are in agreement with the theory.

THEORY

As a starting point for our analytic theory we review some basic results for a semi-flexible homopolymer. The packing arrangement of a semiflexible polymer that optimizes bending within the torus is certainly not a trivial matter and has been treated elsewhere [7]. For simplicity we will assume a simple garden hose or circular packing. We will also assume that the chain enrolls several turns in such a way that it is thick enough to give a well defined toroidal surface, but it is thin enough so that the minor radius of the toroid, r, is much less than the major radius, R. For such a thin toroid, every section of the chain has approximately the same curvature. Using two theorems of Pappus, the surface area and volume of the

toroid are: $A = 4\pi^2 Rr$ and $V = 2\pi^2 Rr^2$. Therefore, the free energy of the chain can be written as:

$$\Delta F = \frac{\kappa L}{2R^2} + 4\pi^2 \gamma Rr \qquad (1)$$

Here the first term represents the bending energy while the second term is the surface energy, $L = Nl$ is the total length of the polymer along the chain, N is the number of repeating units, l is the monomer length, κ is the bending constant, and γ is the surface tension (which is in this model the parameter that characterizes the polymer-solvent interaction).

Using the volume constraint $V = La^2$ (a^2 is the cross section of the chain) the minor radius of the toroid can be written as, $r = \frac{a}{\sqrt{2\pi}}\sqrt{\frac{L}{R}}$ and equation 1 transforms to:

$$\Delta F = \frac{\kappa L}{2R^2} + 2\sqrt{2}\pi\gamma a\sqrt{LR} \qquad (2)$$

If the free energy is minimized with respect to the major radius of the toroid, the following dependence is obtained:

$$\frac{R}{a} = \left(\frac{\alpha}{\sqrt{2\pi}}\right)^{\frac{2}{5}} b^{\frac{1}{5}} N^{\frac{1}{5}} \qquad (3)$$

Here we have used the dimensionless parameters $\alpha \equiv \frac{\kappa}{\gamma a^3}$ and $b \equiv \frac{l}{a}$.

Consider now a more complex problem: a semi-flexible copolymer formed by a sequence of two different immiscible blocks (which we call "block A" and "block B"). For simplicity, an equal size for both blocks will be assumed. If the repeating unit along the chain (that is, the sequence of a block A followed by a block B) is called a diblock, then, for a copolymer, l will denote the length of a diblock while N will denote the total number of diblocks along the chain. The repulsive interaction between blocks A and B is assumed to be dominant over all other interactions such that it may be modeled by imposing the restriction that a block A cannot have lateral contact with a block B. According to this restriction, if the copolymer still forms a toroid in poor solvent conditions, the ratio of the toroid perimeter, $2\pi R$, and the diblock length, l, should be necessarily a whole number; that is,

$$R = m\frac{l}{2\pi}, \quad m = 1, 2, 3, \ldots \qquad (4)$$

Here the number m denotes the number of diblocks per turn in the toroid. The free energy of the toroid still has the same form as in equation 2 and since block types A and B will allways have equal surface areas within our model we can make the interpretation $\gamma \equiv \frac{(\gamma_A + \gamma_B)}{2}$, where γ is the average surface tension of block types A and B. For simplicity we also assume that $\kappa_A \equiv \kappa_B$ so that only a single κ is necessary. We should note however, that the value of the radius R is now restricted to be chosen from a set of discrete values, given by equation 4. Hence the free energy itself varies in a discrete way:

$$(\Delta F)_m = \frac{2\pi^2 \kappa}{lm^2} N + 2\sqrt{\pi m} \gamma a l N^{\frac{1}{2}} \quad (5)$$

The transition from a toroid with $m + 1$ diblocks per turn, to a toroid with m diblocks per turn occurs when the difference in free energy, $(\Delta F)_{m+1} - (\Delta F)_m$, changes from negative to positive. If we define the parameters Υ and f_m as in equations 6 and 7 then this will occur when $\Upsilon \leq f_m$.

$$\Upsilon = \frac{\pi^{\frac{3}{2}} \alpha N^{\frac{1}{2}}}{b^2} = \frac{\pi^{\frac{3}{2}} \kappa N^{\frac{1}{2}}}{\gamma a l^2} \quad (6)$$

$$f_m = \left(\sqrt{m+1} - \sqrt{m} \right) \left(\frac{1}{m^2} - \frac{1}{(m+1)^2} \right)^{-1} \quad (7)$$

In general, for $m > 1$, the toroid with m diblocks per turn will be stable if the condition 8 is satisfied.

$$f_{m-1} \leq \Upsilon \leq f_m \quad (8)$$

When $m \gg 1$ the discrete jumps in R should be small compared to R, so that we should recover the behavior of a homopolymer, given by equation 3. Note that when m is big, we can approximate $f_m \approx \frac{m^{\frac{5}{2}}}{4}$ and the transition points occur when $\frac{m^{\frac{5}{2}}}{4} \approx \Upsilon$. Therefore the radius of the toroid in the transition, given by the restriction 4, becomes $\frac{R}{a} = m\frac{l}{2\pi} \approx \left(\frac{\alpha}{\sqrt{2}\pi} \right)^{\frac{2}{5}} b^{\frac{1}{5}} N^{\frac{1}{5}}$, coinciding with equation 3 as expected.

However, when the parameter Υ is small (that is, when the surface energy dominates over the bending energy) the radius of the toroid will reach its lower limit, $R = \frac{l}{2\pi}$, corresponding to $m = 1$ diblocks per turn. This lower limit to R also implies a lower limit to the surface area within the toroidal geometry. Thus we expect that as Υ becomes still smaller and surface energy even more important the polymer must explore alternative conformations to the simple torus.

One possible conformation, for small values of the parameter Υ, would be a figure 8 conformation, such as that shown in figure . In this conformation a block A enrolls a whole number of turns, n, around a circle of radius ρ in the left side, the subsequent block B enrolls n turns around a circle of the same radius in the right side. and
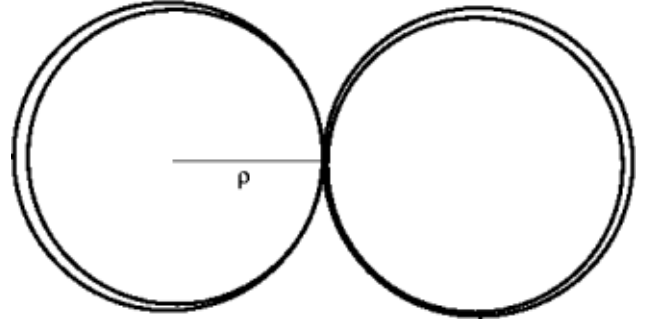

FIG. 1: Schematic diagram of a polymer in the figure 8 configuration with $n = 2$

so on. The free energy of the copolymer in this figure 8 conformation is approximately equal to the free energy of two separated toroids of radius ρ, but each of them with a total chain length $\frac{L}{2}$ instead of L. Therefore, the expression for the free energy, ΔF_8, corresponding to the figure 8 conformation, would be:

$$\Delta F_8 = \frac{\kappa L}{2\rho^2} + 4\pi\gamma a \sqrt{L\rho} \quad (9)$$

Now the restriction of no lateral contact between blocks A and B is imposed. Since each block (of length $\frac{L}{2}$) enrolls n turns (with n a whole number) around the circle of radius ρ, this leads to:

$$\rho = \frac{l}{4\pi n} \quad n = 1, 2, 3, \ldots \quad (10)$$

Lets forget momentarily this last restriction and study the associated problem of a homopolymer that somehow (for example, by imposing some unspecified restrictions) has acquired a figure 8 conformation with free energy given by equation 9. The equilibrium radius, ρ, of both sides of the figure 8 would be: $\rho = \left(\frac{\kappa}{2\pi\gamma a} \right)^{\frac{2}{5}} L^{\frac{1}{5}}$. Substituting this result in equation 9 we obtain a free energy $\Delta F_8 = \frac{5}{2} \left(2\pi a\gamma \right)^{\frac{4}{5}} \kappa^{\frac{1}{5}} L^{\frac{3}{5}}$. Comparing with the analogous free energy of the toroid conformation of a homopolymer, $\Delta F = \frac{5}{2} \left(\sqrt{2}\pi a\gamma \right)^{\frac{4}{5}} \kappa^{\frac{1}{5}} L^{\frac{3}{5}}$, we can readily see that ΔF_8 is always bigger than ΔF by a factor of $2^{\frac{2}{5}}$. Therefore, in the case of a homopolymer, where the value of the radius is not restricted, the toroid conformation is always more stable than the figure 8 conformation. This is not surprising, since there is obviously an increase in bending when going from a torus to a figure 8. As noted previously, however, in the case of a semi-flexible copolymer, for small values of the parameter Υ, i.e. when the surface energy dominates over the bending energy, the toroid major radius is not allowed to be smaller than $R = \frac{l}{2\pi}$, while the radius, ρ, of both sides of the figure 8 conformation can still be as small as needed, as long as they satisfy the restriction 10. Thus for small Υ it is possible for the figure 8 to be more stable than the torus.

The free energy of a figure 8 conformation with n turns per diblock is obtained by substituting equation 10 into equation 9, leading to:

$$(\Delta F_8)_n = \frac{8\pi^2 N n^2 \kappa}{l} + \frac{2\sqrt{\pi}\gamma a l N^{\frac{1}{2}}}{\sqrt{n}} \quad (11)$$

A figure 8 conformation would be more stable than a toroid conformation if the free energy of the toroid with one diblock per turn, $(\Delta F)_1$, is bigger than the free energy of a figure 8 conformation, $(\Delta F_8)_n$ for some value of the number of turns per diblock, n. Using equations 5 and 11, this condition leads to:

$$(\Delta F)_1 - (\Delta F_8)_n =$$
$$= \frac{2\pi^2 \kappa N}{l}\left(1 - 4n^2\right) + 2\sqrt{\pi}\gamma a l N^{\frac{1}{2}}\left(1 - \frac{1}{\sqrt{n}}\right) \geq 0$$
$$\implies g_n \equiv \left(1 - \frac{1}{\sqrt{n}}\right)\frac{1}{(4n^2 - 1)} \geq \Upsilon \quad (12)$$

Note that $g_1 = 0$ and hence there cannot be a transition from a toroid conformation to a figure 8 conformation with just one turn per diblock, $n = 1$. However, for values of the parameter Υ smaller than $g_2 \approx 0.0195$, a transition to a figure 8 conformation with two turns per diblock would be favourable.

Finally, transitions between figure 8 conformations with different values of n can also be studied by analyzing the difference between their free energies, $(\Delta F_8)_n - (\Delta F_8)_{n+1}$. When this difference becomes bigger than zero, the conformation with $n + 1$ turns per diblock becomes more stable than the conformation with just n turns per diblock. It is easy to see that

$$(\Delta F_8)_n - (\Delta F_8)_{n+1} \geq 0$$
$$\implies h_n \equiv \frac{1}{4}\left(\frac{1}{\sqrt{n}} - \frac{1}{\sqrt{n+1}}\right)\frac{1}{\left[(n+1)^2 - n^2\right]} \geq \Upsilon(13)$$

Summarizing the previous discussion the following regimes are expected:

(i) In the case in which inequality 8 is valid, a toroidal conformation with m diblocks per turn (for $m > 1$). The radius of the toroid would be given by equation 4.

(ii) If $g_2 \leq \Upsilon \leq f_1$, then a toroidal conformation with one diblock per turn would be expected. The toroid radius would be given by $R = \frac{l}{2\pi}$.

(iii) If $h_2 \leq \Upsilon \leq g_2$, then a figure 8 conformation with one turn per block is expected.

(iv) Finally, a figure 8 conformation with n turns per diblock may be expected if $h_{n+1} \leq \Upsilon \leq h_n$.

BROWNIAN DYNAMICS SIMULATIONS

As a compliment to the analytic treatment above we have conducted Brownian dynamics simulations with the intention of confirming that structures such as the figure 8 and toroids with $m > 1$ are realistic polymer morphologies, and that their relative energies conform with our theoretical predictions.

In our simulation model, poor solvent conditions were imposed by using a Lennard Jones pair potential between like monomers ie $U_{AA} = U_{BB} = 4\epsilon((\sigma/r)^{12} - (\sigma/r)^6)$, where r is the inter-monomer distance. ϵ is the strength of the interaction and is our unit of energy. σ is roughly equal to the equilibrium inter-bead distance and is therefore related to the parameter a in our analytic work. We chose $\sigma = 2$ for all simulations. For simplicity in our analytic work we specified that blocks of A type monomers and B type monomers cannot have lateral contact. Of course, such a rigid constraint cannot occur for a real polymer and a more realistic situation is that monomers A and B experience a repulsive interaction which offsets their solvent induced attraction. We model this in our simulation by specifying the pairwise excluded volume interaction between monomers A and B as, $U_{AB} = 4\epsilon(\sigma/2r)^{12}$.

In order to account for the stiffness of the chain we used the potential $U_{bend} = -\kappa' cos\theta$ where θ is the angle between successive bonds and κ' is the bending constant. κ' is related to the quantity κ used in analytic work through the relation $\kappa' = \kappa a$.

Bonds between successive monomers were modeled using the potential $U_{bond} = kTd^{-1}\ell^*(r/(d))$ which is equivalent to a Gaussian chain entropic spring potential $3kTr^2/(2d^2)$ at short extensions but increases rapidly for extensions close to or beyond the bond length d. For simplicity, we chose a bond-length equal to the bead diameter such that $\sigma \approx a = d = 2$. We calculated particle motion by integrating the Langevin equation with a time-step of $\Delta t = 0.002$ a temperature of $k_B T = 1$, a frictional coefficient $\zeta = 1$ and random fluctuations $f(t)$ given by a Gaussian distribution with mean 0 and variance $\sqrt{2k_B T \Delta t}$. All simulations were carried out for a total of 25×10^6 time-steps.

Since our intention was to observe striped toroidal structures with different numbers of blocks per turn, m we initially chose to simulate a polymer with a large number of blocks $N = 20$. The total chain length was 400 monomers, which gives $L = 800$ and $l = L/N = 40$. Stiffness was chosen to be relatively large $\kappa' = 15$ so as to avoid globular configurations which are not accounted for in our analytic model. We conducted 40 replicate runs in which a polymer with the above parameters was quenched from an equilibrated configuration in good solvent conditions to poor solvent via the imposition of inter-monomer LJ potentials. We found that after an initial period of collapse, a steady state configuration was reached which showed no change over at least the final half of the simulation time. A wide variety of such configurations were observed, including those with $m = 2, 3, 4$ as well as some previously unexpected mor-
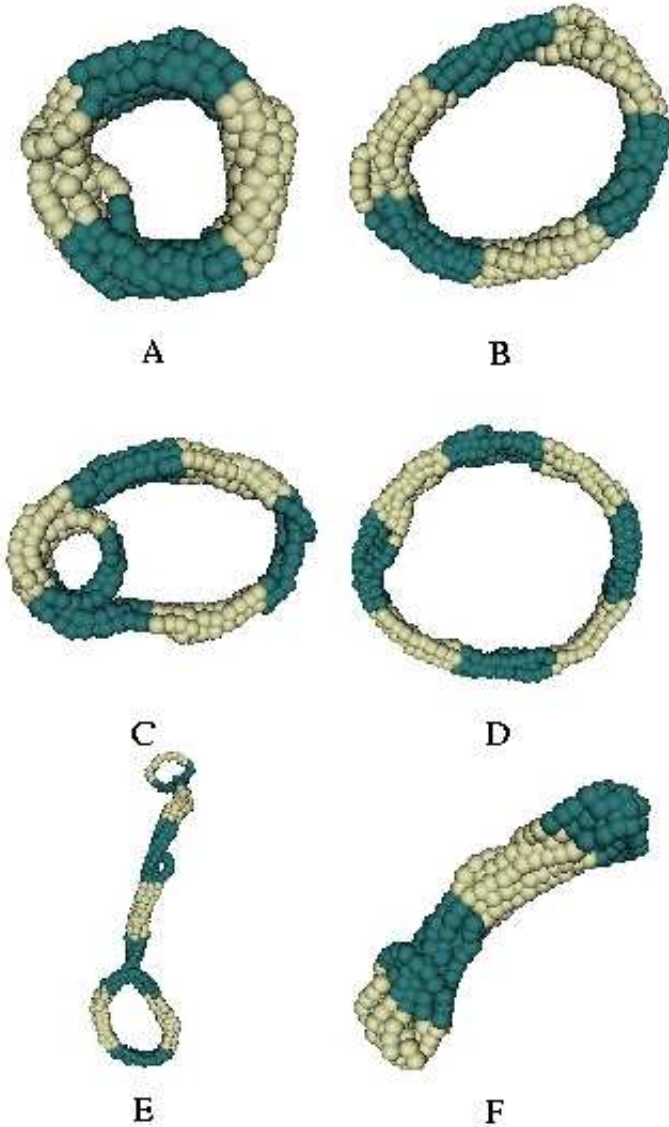
FIG. 2: Steady-state collapsed configurations of semiflexible copolymer where $N = 20$, $L = 400$, $\kappa = 15$. Snapshots shown illustrate the diversity of collapsed states and are labeled alphabetically. The relative internal energies of these states, $U(\epsilon)$ and their frequency of occurrence f as a percentage of the 40 replicate simulation runs are as follows. A: $U = 0 \pm 10$, $f = 20\%$, B: $U = 115 \pm 20$, $f = 10\%$, C: $U = 30 \pm 20$, $f = 20\%$, D: $U = 250 \pm 10$, $f = 12.5\%$, E: $U = 360, f = 2.5\%$, F: $U = 300 \pm 40$, $f = 27.5\%$. Overall values for U and associated errors are calculated from the set of all replicates that collapsed to a particular configuration. Values for each of these simulations in turn were taken over the final 1×10^7 time steps of the simulation.

phologies. Since entropic effects are negligible in our poor solvent system the free energies of each of these configurations are roughly equal to their internal energies U. Under this assumption we found that the $m = 2$ configuration was the most stable of those observed, with all other states including toroids with $m = 3$ and 4 all being higher in energy.

Close examination of the configurations in figure reveals several points of particular note. Firstly, we see that the repulsion between A and B type monomers effectively results in a complete segregation of these blocks. The end result is almost identical to the constraint used in our analytic calculations that A and B blocks cannot have lateral contact. What is not predicted in our analytic theory is that this segregation of A and B blocks leads to the formation of a diverse range of metastable structures. This occurs because A and B type monomers cannot slide against one another, which is an important equilibration mechanism for homopolymers. As a consequence, we find that structures with $m = 3$ and $m = 4$ cannot readily transform to the more stable $m = 2$ type torus. Instead they remain as metastable states. In addition to the striped toroidal states we also observe other interesting metastable structures such as a torus within a torus (figure C) or a torus at the end of a rod (figure E) or a striped rod (figure F). In all cases it seems likely that the A B segregation prevents a transition to a state of lower free energy.

Although the simulation parameter ϵ is clearly related to the surface tension γ, this relationship is very difficult to obtain from a first principles calculation. Instead, we can calibrate γ for our simulation system by using the internal energies of the striped toroidal states. The relative energies of these states should be given by equation 5 so by fitting this equation to values of U and allowing for an unknown additive constant, we can obtain a rough estimate for γ. The value of γ so obtained was $\gamma \approx 0.4$. By substituting this value of γ back into equation 5 we find that the energy of the $m = 1$ torus for our simulated polymer is predicted to be $U \approx 800\epsilon$. Such a high predicted free energy is consistent with the fact that we never observed the formation of $m = 1$ toroids for the $N = 20$ polymer. Our value of γ can also be used to predict an appropriate set of parameters for the observation of figure 8 structures. In making such a prediction we keep the total polymer length L constant, such that $l = L/N$, and vary N. Keeping all other parameters constant we predict that figure 8 configurations should be observed for $N < 6$. Taking a conservative approach to this prediction we simulated a polymer with $N = 2$ using the same methods as were used for the $N = 20$ case.

In figure we present snapshots of steady state collapsed configurations of the $N = 2$ polymer. The most stable observed configuration in this case is the figure 8, however as with the $N = 20$ polymer many metastable configurations were also observed. Indeed the most commonly observed shape was that of a single torus attached to a rod. Other metastable states included complete rods as well as an $m = 1$ toroid and a strange configuration in which a toroid exists between two ends of a bent rod. Although this last configuration is rare it serves to illus-
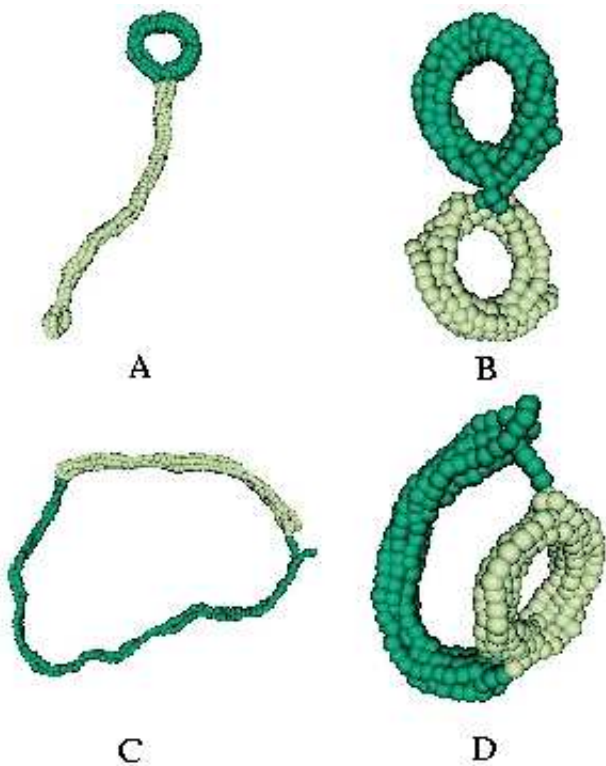
tures in poor solvent. Even the very simple alternating A B block case discussed here forms a range of torii with discrete sizes as well as the unusual figure 8 structure. Aside from this variety of structures one of the key features of these copolymer collapsed states is their well defined size which must be an integer multiple of the block length. Although such structures are yet to be realized we expect that they could have great utility as tools in single molecule experiments, where for example a molecule is pulled through a precisely defined pore (eg torus) [10].

FIG. 3: Steady-state collapsed configurations of semiflexible copolymer where $N = 2$, $L = 800$, $\kappa = 15$. Snapshots shown illustrate the diversity of collapsed states and are labeled alphabetically. The relative internal energies of these states, $U(\epsilon)$ and their frequency of occurrence f as a percentage of the 40 replicate simulation runs are as follows. A: $U = 70 \pm 10$, $f = 37.5\%$, B: $U = 0 \pm 10$, $f = 15\%$, C: $U = 440 \pm 10$, $f = 5\%$, D: $U = 140$, $f = 2.5\%$. Rods $U = 220 \pm 10$, $f = 35\%$ are not shown. See figure for details on calculation of averages and errors

trate the diversity of metastable states which occur when A and B type blocks repel one another. Provided the A B repulsion is sufficiently strong we would expect that many of the the observed metastable states should be quite long lived.

CONCLUDING REMARKS

Semiflexible copolymers exhibit the potential for formation of a wide variety of single molecule compact struc-

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